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13. ABSTRACT (Maximum 200 Words) BACKGROUND: Although numerous studies have defined risk factors for invasive breast carcinoma, few studies have examined those associated with in-situ carcinoma of the breast (BCIS). METHODS: The data include all female cases of BCIS diagnosed among residents of the state of Connecticut from September 15, 1994 to March 14, 1998 (n = 1088) as well as a series of random-digit-dial (RDD) controls selected from the state of Connecticut (n = 1048). Cases are between 20 and 84 years at time of diagnosis while controls are frequency matched to the cases by five-year age intervals. Telephone interviews were used to collect information on family history of cancer, pregnancy and menstrual history, and exogenous hormone use as well as cancer screening history including use of mammography. RESULTS: Eighty-four and twelve percent of cases were classified as pure ductal (DCIS) or lobular (LCIS) histology, respectively. Among women with DCIS, cases were more likely than controls to be older at interview (57.9 vs. 55.9 years) and at age of last menstrual period, as well as more likely to be nulliparous (OR = 1.64, 95% CI = 1.23, 2.19), have fewer pregnancies, have had a screening mammogram (OR = 2.19, 95% CI 1.58, 3.02), have used estrogen replacement therapy (OR = 1.32, 95% CI 1.10, 1.60), have had at least one breast biopsy (OR = 3.98, 95% CI = 3.24, 4.89), and have a family history of breast cancer (OR = 1.51, 95% CI = 1.23, 1.84). The risk of DCIS was decreased for users of oral contraceptives (OR = 0.78, 95% CI = 0.63, 0.96) and for those who had used alcohol in the past year (OR = 0.81, 95% CI = 0.66, 0.98). DCIS cases and controls did not differ by smoking history, race, weight, height, Quetelet's index, or education. Findings were similar for LCIS cases. CONCLUSIONS: These data are the largest prospective case/control study of BCIS to date. The results suggest that many of the risk factors traditionally associated with invasive breast cancer, including a family history of breast cancer, are also associated with the development of BCIS.					
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FOREWORD

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THE GENETIC EPIDEMIOLOGY OF BREAST CARCINOMA IN SITU

5. INTRODUCTION

Breast cancer remains one of the most important health care issues of the 20th century. Despite a wealth of studies on the topic, the current literature provides little information regarding the nature of the epidemiologic risk factors or clinical characteristics of breast tumors which are classified as non-invasive, i.e., breast carcinoma in situ (BCIS). As screening efforts throughout the United States have increased, so has the number of women diagnosed with BCIS, with up to 20% of screened patients diagnosed with this lesion. The identification of risk factors associated with the development of BCIS is especially important, particularly in light of the fact that in the coming century up to one in fifty women in the United States will be diagnosed with this tumor during her lifetime. This project will define risk factors associated with BCIS through the mechanism of a case/control study. The study population includes 1088 cases of female breast carcinoma in situ and 1048 age-matched female controls selected from the population of the state of Connecticut over a 3.5 year data collection period. Cases are between the age of 20 and 84 years at time of diagnosis. The controls are frequency matched to the cases by five year age intervals. Telephone interviews were conducted with the study subjects and collected information concerning family history of cancer, pregnancy and menstrual history, hormone replacement therapy, oral contraceptive use, fertility drug use, as well as sociodemographic variables. In addition, a tissue repository consisting of paraffin-embedded tumor tissue collected from a subset of the cases will be formed. The expression of estrogen and progesterone receptors (ER and PR) as well as two of the most frequently reported oncogenes associated with invasive breast cancer, p53 and c-erbB-2, will be examined in these BCIS cases for the first time in a population-based series.

The goals of this study are as follows:

1. To determine whether there is an association between a family history of breast and/or ovarian cancer and the development of breast carcinoma in situ (BCIS).
2. To determine whether there is an association between additional epidemiologic risk factors, including those traditionally associated with invasive breast carcinoma such as age at menarche, age at first birth, and oral contraceptive use and the development of BCIS.
3. To collect paraffin-embedded tumor tissue for a subset of the BCIS cases.
4. To test for the presence of p53 and c-erbB-2 protein expression as well as estrogen and

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progesterone receptor expression using the methods of immunohistochemistry in the paraffin-embedded tumor tissue.

5. To examine the association between p53, ER, PR and/or c-erbB-2 expression in BCIS tumors with clinical and epidemiologic variables including grade and family history of breast cancer.

6. To develop risk prediction models to be used in defining screening guidelines for women not yet diagnosed with BCIS.

Specific Location of Study

Drs. Claus and Holford have offices located in the Department of Epidemiology and Public Health. Dr. Carter has an office and laboratory located within the Pathology Department. Dr. Badve, who now works with the project as a consultant, is located in the Department of Pathology at Northwestern University in Chicago. The offices of the project director and the director of the Rapid Case Ascertainment Shared Resource are located at 200 College Street, New Haven, CT.

6. BODY

RESEARCH PLAN

The cases were ascertained through the Rapid Case Ascertainment (RCA) Shared Resource of the Yale Cancer Center. The physicians of each eligible case were identified by the RCA. The names of patients and physicians were given to the project director by RCA staff. A letter signed by Dr. Claus and the project director was sent to the physicians requesting permission to send a letter of introduction to the case.

Proto-controls were identified by Northeast Research in Orono, Maine through the mechanism of random-digit dialing. Female residents of the state of Connecticut aged 20-84 who are served by a telephone were eligible.

Those cases approved for contact by their physicians were sent a letter of introduction from Dr. Claus and the project director explaining the project. Controls received a similar letter. Informed consent forms accompanied the letter of introduction and study subjects were asked to return them via the stamped, addressed envelope provided. Approximately 1-2 weeks later an interviewer (either Ms. Sheila Griffin or Ms. Marjorie Jasmin) contacted the potential study subject by telephone. If the potential study subject decided to participate, the interviewer administered the questionnaire over the telephone at the patient's convenience after verbal consent

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had been given for the interview. Subjects who agreed to be interviewed were sent an oral contraceptive picture booklet with an accompanying letter. Subjects were interviewed for approximately 30-45 minutes. Interviews of women with particularly complex family or medical histories took somewhat longer. The questionnaire included questions on family history of cancer, pregnancy and menstrual history, oral contraceptive and other exogenous hormone history, medical history, socioeconomic status, as well as alcohol and tobacco use.

Pathology slides and histologic specimens are collected in the form of paraffin-embedded tumor tissue. Cases who agreed to allow us to retrieve paraffin-embedded blocks were sent an authorization of health information form which we then asked them to return via mail. RCA requests and couriers slides and paraffin-blocks from each of the pathology departments as well as returns the slides and blocks after the laboratory analyses are completed. The blocks are returned to the various hospitals after sufficient material has been removed from them. Alternatively, hospitals may choose to cut material from the blocks rather than send the block itself. The slides are quickly returned after our pathologist, Dr. Darryl Carter, has reviewed them to confirm the diagnosis and perform a uniform histologic review.

Medical records are reviewed to provide details requested in the questionnaire regarding dates of diagnoses or pathologic details of diagnosis. In particular, pathology data are useful in identifying tumor blocks most likely to contain tumor. A stamped, addressed envelope is provided for study subjects so that they may return the authorization for release of health information (for review of medical records and retrieval of paraffin-blocks) via mail. The project director telephoned study participants who did not return the form to encourage them to do so. Replacement forms were sent to women who misplace the original form.

YEARLY REPORT

The personnel on the project has remained stable, with Drs. Claus and Holford continuing to act as Principal Investigator and Co-Investigator, respectively. Interviewing is now complete (our two interviewers, Ms. Sheila Griffin and Ms. Marjorie Jasmin, performed all of the interviews). As our project director position now only requires a 50% allocation, our previous project director, Dr. Meredith Stowe has taken a full-time position as a faculty member at Yale and has been replaced by Mr. Thy Do, M.P.H. (Chronic Disease Epidemiology). Mr. Do has worked extensively with Dr. Claus on a variety of epidemiologic projects and has proved to be a valuable asset to the project. A new director of the Rapid Case Ascertainment Service has been appointed, Ms. Ranji Mehta, M.P.H. Drs. Carter and Badve continue as our two pathologists.

The goals of year five included 1) the completion of the interview process, 2) continued uniform histologic review of slides for each case, 3) continued identification, accession, and immunohistochemical testing of paraffin blocks, and 4) data/statistical analyses. For the study,

1738 cases were identified through the services of the Rapid Case Ascertainment Service. One thousand five hundred and twenty-six of these cases have been verified to be eligible. One thousand three hundred four controls have been identified by Northeast Research, 1281 of whom remain as verified controls. Given our initial sample size estimate of 800 cases and 800 controls, we have greatly surpassed our study goals with respect to sample size and power.

Our physician consent rate for cases has remained high with 87% of cases having a consenting physician. This represents a decrease from our previous number (91%) and is primarily due to changes in the consent procedures at two hospitals (Mt. Sinai and St. Francis Hospitals). At both of these hospitals, no case subject may be approached by a study prior to that case 1) being approached by the consenting physician and 2) having the study explained by that physician. As can be imagined, although the majority of these physicians verbally agreed to contact the women, none of the cases at these two hospitals was actually been contacted by these physicians due to restraints of time and personnel on the part of these physicians. This has proved a difficult problem to overcome despite continued negotiations with the hospitals-our RCA service is now at work to resolve some of these issues for future studies.

When able to approach the study subjects, our case and control response rates remain high; among eligible cases who were contacted by our study, 84% agreed to participate in the interview portion of the study. Among eligible controls who were contacted by our study, 82% agreed to participate in the interview portion of the study.

In addition to the completed interview portion of the study, we continued upon the histologic slide/ paraffin block collection portion of the study. This portion entails obtaining written permission from cases to retrieve the slides/blocks and then physical retrieval of this material from hospitals for review/laboratory analysis. At present, only two percent of interviewed cases have actively refused to allow us to retrieve slides/blocks. The remainder have verbally agreed to allow us to retrieve slides and blocks. Approximately 84% of interviewed cases have given written consent for retrieval of histologic slides while 83% of interviewed cases have given written consent for paraffin-block retrieval (note that the difference between the two numbers indicate the women who did not wish laboratory testing performed on their tumors). The percentage for paraffin-block permission represents a 10% increase from last year (73%), after an active move on our part to match our slide permission rate. Once written permission to retrieve pathology slides has been obtained, RCA requests the slides from the various hospitals. We have had good success with obtaining slides for review with no refusals from hospitals at present although in some instances we have had to travel to the hospital to review slides. Both Drs. Carter/Badve then review all of the slides for diagnosis and select blocks for retrieval and laboratory analysis.

We are also continuing to retrieve blocks for those women who have given permission. Of note, we have asked for and received a one-year no-cost extension for this portion of the study. Over the past year, it became clear that the antibodies initially proposed in the grant for the study

of ER, PR, and HER-2 receptor positivity would soon become unavailable or clinically irrelevant and hence we placed a temporary stop on our staining procedures. Specifically, the FDA has now approved the Dako monoclonal HER-2 antibody for testing of HER-2 (c-erbB-2) positivity. As a result, this product is now the primary new clinical measure of HER-2 positivity and, in fact, is the sole product by which women with HER-2 positive results will be allowed to receive the drug Herceptin. In addition to a change in our choice of HER-2 antibody we have been forced to change our selection of ER and PR antibodies as the manufacturer, Abbott Laboratory, has withdrawn their products in compliance with FDA recommendations. As a result of these two events, we are now utilizing Dako products to test for ER, PR, and HER-2 positivity (as well as re-analyzing the previously completed 250 cases with the new products to assure uniformity of data results). Because of the great value of our data set we felt that the need to generate valid and clinically relevant data outweighed the time delay in the completion of this aspect of the study.

Data analysis continued this year and two manuscripts are completed: "The epidemiology of breast carcinoma in-situ" and "Family history and the risk of breast carcinoma-in situ". Results from the first will be presented at the DOD meeting in Atlanta in June 2000.

HUMAN SUBJECTS

Subject Population

All female Connecticut residents between the ages of 20 and 84 years at time of diagnosis and diagnosed with breast carcinoma in situ from 9/15/94 to 3/14/98 were eligible. Cases with a previous history of breast cancer and/or a breast biopsy of unknown outcome were excluded. In this time period, 1738 women were diagnosed with BCIS in the state of Connecticut within the age-group of interest. From this group, we interviewed 1088 women. Proto-controls were randomly selected by an external firm (Northeast Research) and consist of age-matched Connecticut female residents. We identified 1304 proto-controls and interviewed 1048 as controls.

Risks/Benefits

As this is primarily an interview study, we anticipate no physical risk to study subjects. However, given the serious nature of breast cancer, it is conceivable that some patients will experience some degree of psychological distress as a result of being interviewed concerning their health status. In order to minimize the occurrence of such distress, interviewers are trained to conduct interviews in a relaxed, friendly, and professional manner. Swift corrective action will be taken concerning any interviewer whose demeanor seems to have a negative effect on study participants.

There are no monetary inducements to participants in this study. The primary inducement

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for participants is the ability of the study to contribute to our understanding of breast cancer. This research has the potential to define modifiable risk factors associated with the development of breast cancer as well as the potential to identify currently healthy women at increased risk of this disease who might benefit from increased screening for breast cancer.

At present no adverse effects have been reported in this study. A number of positive effects have been reported, particularly to our interviewers, including the improvement of family relationships in association with the gathering of family history information. In addition, among cases, the discussion of a breast cancer diagnosis with an independent observer has proved to be helpful to a number of women.

Protection of Subjects

Each study subject is assigned a code number. The interview cover sheet containing identifying information is removed from the interview booklet and stored separately. All staff members are informed prior to employment and at regular intervals as to the necessity for keeping all data confidential. All written study material is stored in locked file cabinets. All histologic specimens will be stored in the laboratory of Dr. Carter.

The opinion of Dr. Carter, the study pathologist, concerning histologic specimens may in some instances differ from that of the original pathologist. If Dr. Carter interprets the woman's cancer to be invasive rather than solely in-situ, the original pathologist and surgeon will be contacted and informed of the opinion of the study pathologist. If the original pathologist is not available, we will inform the Chair of Pathology at the appropriate hospital.

No information that identifies an individual subject will be given to third parties, including family members, unless that subject has given consent to do so. Information obtained during the study will not be placed in a subject's medical record. Publication and presentation of results will contain only aggregate data.

No laboratory test results on specimens will be released to the participant or her physician. This current work is in the realm of research and any results should be regarded as preliminary findings and not definitive. None of the materials collected on these patients will be used to do research unrelated to their breast cancer diagnosis.

Human Investigation Committee Approvals

We have had great success in obtaining the approval and participation of the state's hospitals. At present, all but four of the state's 35 hospitals are active participants. We are able to identify cases diagnosed and treated at these four hospitals via the Connecticut Tumor Registry. Overall,

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the response of the state's hospitals and medical personnel has been extremely positive. Most of the hospitals are now in their fifth year of participation with our study.